BORNA DISEASE; A LITERATURE REVIEW

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SUMMARY

Borna disease (BD) is an infectious viral encephalomyelitis and meningitis naturally occurring in sheep, horses, and some domestic rabbits. A cyclical and sporadic disease recorded since the beginning of the 19th Century in southern Germany, BD has been consistently diagnosed only in this endemic area.

Symptoms of the disease in the horse are similar to those of the equine encephalitis of the Western Hemisphere. Means of spread and pathogenesis in nature are unknown. Various domestic and laboratory animals are susceptible to experimental infection. Most work has been done with the domestic rabbit. With rabbits and other species, the incubation period with intracerebral (i.c.) inoculation of the virus is variable and may be several months. Subclinical infections have been described. It is assumed that the disease is contagious and that inapparent carriers exist in nature. No gross lesions are seen in natural or experimental cases. Microscopic lesions are confined to the nervous system. Complement-fixing and fluorescing antigens are found in the infected brain. There is no test for systemic antibodies considered reliable for diagnosis. The virus can be grown in tissue culture. Recent emphasis has been on its use as a model for slow virus infections.

SYNONYMS

Enzootic encephalomyelitis, Meningo-encephalomyelitis, Bornasche Krankheit, Genickstarre (cerebrospinal meningitis), Gehirn-Ruckenmarksentzündung der Pferde (brain and spinal cord inflammation of the horse), Nervenkrankheit (nerve disease), Kopfrankheit (head disease), Encephalitis lymphocytaria.

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HISTORY AND OCCURRENCE

Although the Borna disease (BD) was probably described in the beginning of the 19th Century in what is now southern Germany, Borna is the name of a locality in Saxony in which a particularly severe epidemic occurred in 1894 through 1896. BD continues to be endemic in the West German states of Bavaria, Hessia, and Baden-Württemberg, and the East German states of Thuringia and Saxony.

Borna disease is considered sporadic but certainly not rare within the endemic area. In 1896 in Germany, 1,198 horses were reported sick with the disease (Nicolau and Galloway, 1928). In 1932, there were 280 cases reported from Baden-Württemberg and from Saxony, almost 500 (Anonymous, 1934). Between 1953 and 1962 in Thuringia, the disease was diagnosed by histopathology in 1,995 horses and 310 sheep (Karasek, 1963). In necropsies of 4,522 sheep from 1955 to 1963, BD was listed as the most common cause of death, accounting for 23.3 percent of the diagnoses (Seffner, 1966).

In the German Federal Republic, disease reporting is decentralized and systems vary according to the State (Land). In Bavaria, where apparently the reporting of the disease is compulsory for equines only, the disease incidence in horses for the period 1939-48 was 0.7 percent, in the years between 1949-58, 2.4 percent, and from 1959-68, 0.5 percent (Wagner, 1970).

Controversy has always existed on the possible presence of BD outside of the endemic area. This question is still not resolved. Since it was early recognized as a distinct entity, encephalitides throughout the world, particularly of equines but in all animal species, have been compared with the Borna disease.

In Germany, the presence of intranuclear inclusion bodies in the central nervous system (CNS), called Joest Degen (JD) bodies, are considered pathognomonic for the disease. To pathologists, this lesion is the prototype for the type B intranuclear inclusion body (Prier, 1966). Inclusion bodies described as similar to or indistinguishable from JD bodies, however, have been found in various disease conditions elsewhere, including some of the American equine encephalitides (Innes and Saunders, 1962).

In Germany, the disease signs, in equines at least, may be considered characteristic. Elsewhere, as they are reviewed in "clinical features," signs of this disease would be easily confused with those of other encephalitides.

Howitt (1937) established that BD virus (BDV) did not react serologically with Eastern or Western equine encephalitis sera (EEE, WEE). Few are the reports from anywhere, which are accompanied by serological comparisons. Until comparatively recently, BDV was difficult to identify by serological means (von Sprockhoff, 1954b). Daubney and Mahlau (1957) described Near Eastern equine encephalomyelitis (NEEE) of donkeys, horses, goats, sheep, and cattle in Egypt and Syria, which was subsequently shown probably not to be EEE, WEE, VEE (Venezuelan equine encephalomyelitis), St. Louis,

Japanese B, and West Nile, on the basis of a survey of animals in infected areas (Sabban and others, 1961). Also, Daubney and Mahlau (1967) published a detailed description of NEEE, confirming it to be insect-borne. Daubney (1967) described comparative work with NEEE and four strains of BDV from Germany, in which the two viruses behaved identically in his hands with regard to animal susceptibility, lesions, and characteristics of tissue culture growth. Immunological comparisons between the two were inconclusive and serological identification was not attempted.

Reports from throughout the world, describing the disease as "identical to," "related to," or "distinct from" BD, may or may not be backed up by histopathology or serology to support claims. In some of these reports, there is no way for the reviewer of the literature to judge whether it is or is not Borna disease that is being described.

Very likely BD exists elsewhere than in southern Germany, but it only is there that BD has been consistently diagnosed and studied. The great bulk of published material is from German sources.

CLINICAL FEATURES

In the equine, BD is seen as a severe CNS disorder, which could be confused clinically with other neurotropic viral diseases.

Mayr and Pette (1968) described the classic symptomatology as follows: "Borna begins with a disturbance of general health, through gastroenteritis and respiratory distress. Then come meningoencephalitic disturbances, which can be very distinct--excitement and depression, uncertain step, spastic muscle contraction, excessive salivation, nystagmus and paresis. A well-known characteristic is abnormal stance. Forced movement, cramps and sudden collapse vary the clinical picture."

In a resumé of more than 500 treated cases, signs noted most frequently were inappetence, constant chewing, priapism, head drooping, frequent lying down, unsteady gait, muscular contraction, colic, bending of neck to the side, and grinding of the teeth (Goerttler and Vöhringer, 1954). Impaired vision is frequently described, with pupil reaction to light delayed or absent. Congestion of retinal blood vessels is seen (Walther, 1952; Müller and Fritzsch, 1955).

In the horse already showing neurological signs, temperature elevated to 103° F is noted in some cases only. High temperature is not maintained throughout the disease's usual 1 to 2 week course. Neither respiratory nor pulse rate abnormality are constant features of the neurological stage of the disease (Heinig, 1969).

The mortality rate in equines is reported to be between 75 and 90 percent. One report stated that 60 of 106 infected horses treated with sulfonamides recovered (Wagner, 1951). Another claimed a cure rate of 6 of 9 horses treated with iodides, sulfas, and hexamines (Mäder, 1952), and

yet another, a 45 percent cure rate of 501 infected horses with sulfonamide therapy (Goerttler and Vöhringer, 1954). From these reports, it may be surmised that secondary bacterial infection accounts for the high reported mortality rate or that a CNS disease of other origin may be reported as caused by BD in the endemic areas.

After-effects in those equines surviving infection are described as hydrocephalus internus, "weak back," blindness, and motor disturbance. Relapse can occur (Heinig, 1969).

The incubation period of the disease in nature is unknown but is presumed to exceed or at least equal the average of 6 to 7 weeks following i.c. inoculation (Heinig, 1964).

The clinical features of BD of the horse are described as remarkably similar to those of the American arthropod-borne equine encephalitides.

Sheep also sicken an average of 6 to 7 weeks after experimental i.c. infection, and the disease course is 1 to 2 weeks (Heinig, 1964). Sheep may show a typical encephalitis, with depression, unnatural stance, hyperexcitability alternating with lethargy, eventual paralysis, and death. Spontaneous recovery, as in the equine, may occur. Mortality is described as approximately 90 percent.

Another form of the disease in sheep is frequently described, which is characterized by repeated and sudden clonic convulsions. Several times a day the affected animal may collapse and lay motionless for periods of up to a few minutes. Between attacks the animal behaves almost normally. Ihlenburg (1959) described this clinical picture in 7 of 80 cases in sheep, with recovery in 3 of the 7. Matthias (1954) and Ihlenburg (1957) believed that sheep may be subclinical carriers of the disease.

The rabbit has long been considered a good laboratory model for BD but has recently been found to be a natural host. Otta and Jentzsch (1960) described a spontaneous outbreak in rabbits in an endemic area. This outbreak has been followed by documentation of seven others from 1967 to 1969. Disease signs in these outbreaks include inappetence, emaciation, apathy, somnolence, inability to hold head straight and other postural difficulties, gnashing of teeth, and staggering, which is sometimes circular. The course of the disease was usually 3 to 7 days. The seven outbreaks were all confirmed histopathologically and two of these also by transmission and complement-fixation tests (Johannsen and Bergmann, 1971).

Nicolau and Galloway (1928), who observed more than 200 experimentally infected rabbits, described the first sign as depression. After a slow weight loss, the animal shows incoordination in righting itself after being placed on its side. Somnolence, blindness, grinding of the teeth, and increased salivation ensue. There is paresis of the ears and hanging of the head, followed by a progressive paralysis, first of the hindlegs then the forelegs. These authors never observed excitement in affected

animals but always depression. Another observer of infected animals described hypersensitivity with occasional tetanic convulsions. Inability to lift ears was described as often one of the first visible signs (Shadduck, 1971).

The goat has been reported naturally infected with, as is unusual in sheep, the development of a circling walk. In goats experimentally infected (Ihlenburg, 1962), adults seemed refractory to i.c. infection, with only one of six showing transient nervous signs. Attempts were negative at viral isolation from their brains. Two of four kids inoculated developed signs, and the virus was isolated. One died immediately after onset of nervous signs and another showed muscular tremors, opisthotonos, and staggering gait.

In one clinical study of 183 goats necropsied with diagnosed CNS disorders, only 6 had nonsuppurative encephalitis characteristic of BD, leading to the conclusion that goats are probably unimportant hosts of the disease (Heinig, 1969).

Cattle have occasionally been reported to be infected with BD, with most reports in the early literature (Nicolau and Galloway, 1928; Ziegler, 1933). Matthias (1954) isolated BDV from two asymptomatic calves 40 and 139 days post i.c. infection, and concluded that bovines may be inapparent carriers of the disease.

Malignant catarrhal fever in bovines caused by BDV had long been suspected mostly on the basis of early work (Nicolau and Galloway, 1930) or some field observations (Beltrami, 1940). Matthias (1960) published results of i.c. infection of 58 calves. He was able to cause nervous system symptomatology in eight of these calves only by stressing them weekly with intravenous (i.v.) injection of Escherichia coli (E. coli) vaccine. None of the animals showed symptoms or lesions of malignant catarrhal fever.

Swine have not been reported sick with BD nor have infection attempts with this species been successful (Matthias, 1955a).

PATHOLOGY

No gross lesions are observed consistently in the necropsy of either natural cases or artifically infected animals. Microscopic lesions are confined to the nervous system of affected animals and these are primarily in the CNS. These lesions fall into the category of meningitis and encephalomyelitis with inclusion bodies.

Acute disseminated inflammatory changes occur throughout the brain and spinal cord. The midbrain has been described as being the most severely affected, and in the horse particularly, gray matter around the aqueduct and in the substancia nigra. Other sites of predominance in the brain include the caudate nucleus, Ammon's horn, the pyriform area, the

medulla at the floor of the fourth ventricle, and the nuclei of cranial nerves V, VI, VIII, IX, and X (Seifried and Gylstorff-Sassenhoff, 1958).

The nervous system is affected similarly as with most viral diseases. There are perivascular and focal infiltrations of lymphocytes and plasma cells, and neuronal degeneration with neuronophagia (Innes and Saunders, 1962). Gliosis is also seen. The meninges are irregularly affected with focal infiltration of lymphocytes and histiocytes around meningeal vessels. Infiltrations are found around vessels of the brain and spinal cord, with post-capillary venules of the brain most severely and consistently affected.

Gliosis is an early-occurring prominent feature of Borna disease's histopathology. Diffuse gliosis and focal nodules of proliferating microglia, often in association with perivascular cuffs, are seen.

Degenerative changes of ganglion cells are most widely distributed than inflammatory changes. Neuronophagia is found regularly in the medulla oblongata and midbrain, as well as in the spinal and paravertebral ganglia. In the progress of the disease, the nuclear membrane disintegrates and division between cytoplasm and nucleus becomes obscure. The nucleus and nucleolus stain poorly and numerous small intensively stained granules are seen.

Acidophilic intranuclear inclusion bodies in ganglion cells, JD bodies and considered pathognomonic for the disease in Germany, are often seen in brain histopathology. These range from 0.1 to 3 microns in diameter and are occasionally seen in the cytoplasm. They are usually found surrounded by a clear halo, and some have internal structure similar to the Negri body of rabies. They stain lightly red by the Lentz method and are Feulgen-positive. Often more than one inclusion body can be found per cell (Seifried and Gylstorff-Sassenhoff, 1958).

The predominent site of JD bodies is Ammon's horn. They are found frequently also in the caudate nucleus and the olfactory bulb. They may be seen throughout the CNS. The cerebellum has been described as the least likely site for these lesions or viral antigen (Shadduck, 1971). Immunofluorescence is found also only in neurons, and concentrated in nuclei where JD bodies occur. This points to the conclusion that ganglia are the primary site of virus replication (Shadduck and others, 1970). These inclusion bodies may not be found in brains of animals that die of the disease (Cohrs, 1967). Joest Degen bodies are not consistently found in known infected animals. They have been shown to occur at times, however, in all species clinically susceptible to infection with the virus.

In the first electron microscopic study of the infected rabbit brain, filamentous bundles of possible viral origin were occasionally seen in central and peripheral neurons. Also seen were intracytoplasmic crystalline aggregates in non-neuronal cell elements of the central and peripheral nervous system. The latter lesions were considered probably of nonspecific character (Anzil and Blinzinger, 1972; Anzil, 1972).

In EEE and WEE, the location of lesions may be different than in BD. Macroscopically observed changes in the nervous system and other organs are usually seen. Histologically, nervous system reaction is similar: lymphocytic infiltration, neuronal degeneration, and glial proliferation. Hyperemia and petechiation of the brain or the spinal cord are commonly found in EEE and WEE but not BD. The same is described with foci of rarefaction necrosis. The presence of neutrophils in lesions predominates in EEE. Unlike in BD, the fifth cranial nerve is not involved in EEE and WEE (Innes and Saunders, 1962).

Inclusion bodies very similar to JD bodies have been seen in some cases of EEE, WEE, and R (Russian) EE and even possibly equine infectious anemia (Innes and Saunders, 1962). The inclusion bodies that are caused by NEEE isolates could not be differentiated from those of BD (Daubney, 1967). A pathologist who has worked with BD has observed that JD bodies could be confused with normal features of the dog, cat, and sheep brains. Under routine brain histopathology procedures in the United States, intracytoplasmic JD bodies might be mistaken for Negri bodies of rabies infection (Shadduck, 1971).

PATHOGENESIS

Most work with this virus has been done by i.c. inoculation of laboratory animals. An early assumption not yet discarded was that the virus travels centrifugally from the CNS along nerve routes to peripheral nerves and glands of the animal.

Nicolau and Galloway (1928) found that by either intracranial or parenteral inoculation, a "descending neuritis" in peripheral nerves occurs which is more intense at the origin than the termination of the ganglia. In inoculation of monkeys, they arrived at the same conclusion that the agent travels from the CNS to the peripheral nervous system along nerves.

Zwick and others (1926), Ernst and Hahn (1927), and Nicolau and Galloway (1928) all demonstrated that infectious virus is eliminated in the saliva and nasal secretions of infected rabbits. Joest had earlier concluded, from spread of the disease in the brain, that the virus reaches olfactory bulbs by the lymph paths of the olefactory region from the nasal cavities and spreads caudalwards (Cohrs, 1967).

Galloway (1930) succeeded in infecting rabbits by intranasal infection. Matthias (1955a) reconfirmed intranasal infection of rabbits and succeeded by this route to infect 3 of 12 sheep. He failed to infect 7 horses by this method.

Nitzschke (1963) was able to infect rats intranasally, but the dose necessary to do this was 130 times that needed for i.c. infection of this species. Heinig (1964) repeated intranasal infection of sheep and was successful in 2 horses by this route. He found nasal secretions of horses

to be infectious before the appearance of clinical disease. Inspiration of infective material is considered a likely method of virus transmission.

Zwick and others (1926) were able to infect rabbits by forced feeding. Von Sprockhoff (1958) was able to infect only one of three baby rabbits by this route. Matthias (1955a) was unsuccessful in infecting rabbits, sheep, or horses by oral spray or use of stomach tube. The likelihood of oral transmission of BD in nature is open for interpretation.

The presence of infective virus in the urine and milk of infected animals was reported by Zwick and others (1926) and is considered a possible means of virus spread.

The presence of BDV has also been demonstrated in the vitreous body of the eye, the submaxillary gland, parotid glands, adrenal glands, and pancreas (Galloway, 1930).

In addition to the i.c., nasal, and oral routes of infection, rabbits have been successfully infected by injection ontraocularly, intraperitoneally, intratesticularly, and by injection into the sciatic nerve. The same investigators were successful in infecting rabbits on the scarified skin only when animals were "prepped" by intracranially injecting saline (Nicolau and Galloway (1928). Nicolau and Galloway (1930) later showed that suckling rabbits were fully susceptible to intraperitoneal and subcutaneous infection.

Route of inoculation apparently has no effect on the incubation period of the clinical disease. Heinig (1958) inoculated rabbits by suboccipital, i.c., and the intranasal routes. He found infective virus and complement-fixing antigen in the brain earlier in i.c. and suboccipital inoculates than in those inoculated intranasally, but animals infected by the various routes sickened the same time postinfection. Rabbits inoculated in the sciatic nerve also showed the same disease incubation period as those infected i.c. (Anzil and Blinzinger, 1972).

The possibility of transplacental passage of the virus has been described. In early works, brains of two foals born of infected mares showed characteristic lesions. Injection of the foal's brains i.c. material in rabbits resulted in BD (Nicolau and Galloway, 1928). Ihlenburg (1957) in later work with rabbits concluded that transplacental infection was improbable. Fetuses of pregnant rats were apparently unaffected by maternal infection (Nitzschke, 1963).

The search for a viremia in BD has been long and results inconstant. Ernst and Hahn (1927) were successful in isolating virus from the blood of rabbits, in some states of the disease, while Zwick and others (1926) were unsuccessful with horses. Later investigators failed to find blood virus in any infected species (Schmidt, 1952; Matthias, 1953; Matthias, 1955b).

The strictly neuronal pathogenesis failed to satisfy all observers of the disease. Heinig (1958) demonstrated that i.c.-infected rabbits

simultaneously showed virus in the brain, cerebrospinal fluid, and nasal secretions 3 days' postinfection. In two inoculated rabbits, blood virus was found.

Later he was able to consistently demonstrate Borna virus in the blood of i.c.-infected rabbits 20 through 72 hours' postinfection. This early presence of virus in the blood would explain the appearance of virus in all parts of the CNS, in the nasal mucosa, and in nasal secretions 3 days after infection (Heinig, 1961).

The pathogenesis of BD in naturally occurring cases is still unknown; possibly the virus spreads along nerve routes from the portal of entry, or the viremia occurs as a phase of the disease. Not to be overlooked is a combination of both means of spread, as may occur in rabies.

VIRUS

Early filtration work established the size of BDV as between 85 and 125 millimicrons (Elford and Galloway, 1933). Electron microscopy of infected rabbit brains has not yielded virus ultrastructure. Neither has its nucleic acid content been discovered. It is considered to have an envelope (Mayr and Danner, 1972a).

The virus is resistant to wide pH variation. It maintains infectivity after 30 minutes' exposure to pH between 5 and 12 (Heinig, 1955). Disinfectants relying on alkalinity are considered ineffective (Heinig, 1969).

Borna disease virus is not particularly heat resistant. Infected brain emulsion heated at 57° for 30 minutes loses virulence (Galloway, 1930). Dessicated btain material sealed in tubes and exposed to light maintained virulence for rabbits for 1 year (Nicolau and Galloway, 1931), but direct exposure to ultraviolet (U.V.) light for 5 minutes inactivated the virus (Nicolau and Galloway, 1928). Heinig (1955-56) later confirmed U.V. light effect on infectivity, but found no parallel influence on complement-fixing activity of exposed virus.

A one to 10,000 solution of potassium permanganate would not destroy virus after 3 hours' exposure (Zwick and others, 1937). Phenol has been ineffective against the virus. At room temperature and 24 hours' exposure to a 0.05 percent solution of betapropiolactone and formalin failed to inactivate virus (Heinig, 1969).

The virus has been quite resistant to environmental influences. Dried brain material has maintained infectivity for at least 17 years (von Sprockhoff, 1953). In physiological saline, BDV survived 85 days, and in horse urine, for 22 days (Zwick and others, 1937). In tapwater at ambient temperature, the virus remained viable for at least a month, and in milk for 3 months (Heinig, 1969).

The virus is complex and centrifugation at 25,000 or 82,000 G's separates a soluble complement-fixing antigen which is noninfective for rabbits (von Sprockhoff and Nitzschke, 1955). This antigen's size is between 15 and 30 millimicrons as determined by ultracentrifugation at 145,000 G's (von Sprockhoff, 1958).

The soluble antigen retains its complement-fixing activity for 1 or 2 days at room temperature and at least 19 weeks at 2° C. Complement-fixing activity was lost at 1 month at -18° C (von Sprockhoff, 1958). Loss of complement-fixing titer is seen by heating the soluble antigen at 60° C for 30 minutes (von Sprockhoff, 1955).

Glycerine may not be the ideal preservative for infective material when data are compared with those of dessicated brain. At ambient temperature the virus has retained virulence for 6 but not 12 months in 50 percent glycerine. At 4° C in glycerine, the virus lasted at least 200 days (Galloway, 1930). Later studies have found erratic survival of virus in glycerine (Heinig, 1969). Lyophilization of infected brain material has been used successfully (Daubney, 1967).

For disinfection in the endemic zone, formalin and chlorine base disinfectants have been employed. Formalin concentration should be strong for effective use. Chlorine disinfectants have inactivated virus completely in practical concentrations within minutes (Heinig, 1969).

IMMUNITY

The disease has long been considered to propagate along nerve routes and the role of humoral antibodies is unknown. No serotypic variants of the BDV have been described.

Early work with rabbits revealed that intracerebrally inoculated animals could be protected by periodic i.v. injections of the virus during the incubation period. Rabbits given i.v. virus periodically would also withstand subsequent i.c. challenge exposure (Nicolau and Galloway, 1928).

Changes in virulence of the virus by animal passage have been noted. Intracerebral passages in sheep are reported to reduce pathogenicity for horses and the virus of higher i.c. passage is less pathogenic for sheep than that of lower ones (Heinig, 1964). As may be expected, increased virulence may also occur with i.c. passage. In rats, continuous passage decreased incubation and survival time considerably and increased infective titer of brain suspension (Nitzschke, 1963).

Attenuation through horse passage of infected brain material is the source of one vaccine strain used in Germany. Three subcutaneous inoculations are required for immunity in horses (Wagner, 1970).

Zwick and others (1927) reported immunizing three horses by subcutaneous

inoculation with dried infected rabbit brain material. The horses proved resistant to subsequent i.c. challenge, while two controls succumbed (Galloway, 1930). The Zwick vaccine is used primarily in Germany today. One subcutaneous injection is employed.

Vaccination results of sheep with the Zwick vaccine are considered good, with one dose claimed to provide immunity for in excess of a year (Heinig, 1969).

Assessment of field vaccination data with this cyclical is widely accepted and sporadic disease is difficult. Two such studies in equines have produced inconclusive results. In one report from Bavaria in 1952, the disease developed in 0.4 percent of vaccinated horses as opposed to an incidence of 3.1 percent in the unvaccinated population (Rauscher, 1959). In another study, 2,300 horses were vaccinated and compared with 2,500 controls of the same district. Of the vaccinates 20 sickened with BD while 29 of the nonvaccinates became infected (Möhlmann and Maas, 1960).

Whether animals that have survived infection become permanently immune is not known. Relapses of the disease have been reported (Heinig, 1969).

EPIDEMIOLOGY

The epidemiology of BD is far from being understood. The cyclical nature of the disease has been described. In one study covering the period of 1953-1962, in which the disease was confirmed by histopathology of 1,995 horses and 310 sheep, the investigator stated that disease incidence increases every third year, affecting mainly 3- to 6-year-old horses. Sex had no influence on susceptibility. The geographical distribution of the disease was the same for horses as for sheep (Karasek, 1963).

Young animals are generally described as being more susceptible than are older animals. The incidence in farm horses is greater than those maintained in town (Heinig, 1969).

Many attempts have been made to demonstrate inapparent disease. These must be interpreted in light of the reported variability of incubation period in experimentally infected animals. In one report, one infected guinea pig was 363 days in coming down with clinical disease, although the average incubation period in this species is less than 2 months (Galloway, 1930). Infected hamsters showed no clinical or pathological evidence of disease for up to 309 days postinoculation (p.i.) (Anzil and others, 1973). Heinig's (1964) reported average incubation period of 6 to 7 weeks in i.c.-infected horses and sheep showed a range of 24 to 143 days.

On the basis of lesions and viral isolation from i.c.-infected horses, sheep, and calves, some of which showed no symptoms, Matthias (1954) concluded that inapparent reservoirs of disease exist. In one search, encephalitic lesions were found in 10 of 83 apparently healthy horses

sent to slaughter from farms having had BD the previous year. Viral isolation in rabbits was made from one of these cases (Ihlenburg and Brehmer, 1964).

The finding of lesions or virus in healthy appearing animals may represent true subclinical disease or cases which would eventually show symptoms. It is, however, widely accepted that inapparent carriers of BD exist.

Disease in other than the commonly infected animals present in the endemic area have been searched. The dog apparently resisted infection (Nicolau and Galloway, 1928). The cat has been successfully infected (Ihlenburg, 1966). Some workers have succeeded in infecting chickens (Nicolau and Galloway, 1928; Matthias, 1955a; Ludwig and others, 1973). Wild mice caught in infected stables failed to yield virus (Ihlenburg, 1957). At least one naturally occurring case in Roe deer has been reported (Nicolau and Galloway, 1928).

A survey of complement-fixing antibody prevalence was done in Bavaria to determine the extent of infection in livestock and humans. Complement-fixing antibodies for BD in livestock have not been consistently found, nor of long duration, nor of high titer. No other serological technique, however, is known to be better.

Of 247 horses, 978 sheep, 146 cattle, 115 pigs, and 81 human beings, positive reactions occurred in 2 horses, which had been vaccinated 4 months before the sampling, and in 4 sheep in 2 flocks, which were asymptomatic. Of special public health interest in this survey was that the 81 human sera were from people with diagnosed nervous system disorders (Wagner, 1970).

Long use of the rabbit brain vaccine has conceivably had a role in the maintenance and spread of the virus in the endemic area.

The apparently long incubation period of the disease in livestock does not suggest insect transmission as a probable means of spread. Matthias (1953), failing to isolate virus from the blood of infected horses, concluded that bloodsucking insects play no role in the disease's transmission. Later studies on rabbits do not exclude viremia as a possible phase of the naturally occurring disease (Heinig, 1961).

Some field data point suspiciously toward a possible arthropod-borne transmission of BD. The disease is cyclical in nature. In Bavaria, BD is not found in altitudes of over 700 meters, a characteristic common with arbovirus disease (Wagner, 1970). Although cases occur throughout the year, peak months of incidence in infected zones are in the spring and early summer (Matthias, 1953; Wagner, 1970).

In Germany, BD is considered a Bodenseuche-soil borne disease. Probable mechanisms for its transmission by inhalation and possibly ingestion of infective secretions and excretions have been discussed, along with the

resistance of the virus to the environment.

DIAGNOSIS

Within the endemic area, BD is diagnosed clinically and confirmation is by histological examination of the CNS. The finding of a glial cell proliferation, neuronal degeneration, and lymphocytic infiltration in the brain of a clinically suspicious animal may be considered positive.

The detection of JD inclusion bodies is considered specific but not essential for histopathological confirmation, since these inclusions are not consistently found in infected animals.

Identification of the causative agent may be done in Germany. In light of the similarities of lesions of this disease with others, there would be a necessity for diagnosis outside the endemic area.

Inoculation of the homologous species with suspect infected brain material should be by the i.c. route since this is the only reported consistent manner of reproducing the disease in livestock. Younger animals are considered more susceptible than mature. A long incubation period should be expected with inoculation of sheep and horses (Heinig, 1964).

The rabbit is the laboratory animal of choice for i.c. inoculation of BDV. The incubation period in adult rabbits inoculated i.c. usually ranges from 3 to 8 weeks, with some much longer periods reported (Nicolau and Galloway, 1928; Mayr and Danner, 1972a). The course of the disease is usually 3 to 7 days (Johannsen and Bergmann, 1971). Symptomatology has been described elsewhere. Lesions found in i.c.-infected animals are similar to those found in naturally occurring cases. The incubation period in rabbits may be shortened considerably with use of suckling rabbits 1/2 to 5 days of age, to an average of 12 to 13 days (von Sprockhoff, 1956c).

The rabbit is also the animal of choice for production of immune serum and has been used since 1954 for this purpose. Complement-fixing antibodies can be produced to a serum dilution of up to 1:256 by subcutaneous immunization and subsequent i.c. infection of rabbits (von Sprockhoff, 1954a).

Complement-fixing antigen may be detected in rabbit brain before suspensions of the same material are infective, i.e., at 2 weeks p.i. in adult rabbits (von Sprockhoff, 1955), and at 10 days p.i. in baby rabbits (Heinig, 1961). In baby rabbits, brain may give positive titers at dilutions of between 1:64 and 1:256 (von Sprockhoff, 1956c).

Infective virus may be isolated in animal brains before the onset of signs (Heinig, 1958). Brains from rabbits already showing signs contain infective virus and in moribund animals the titer may be variable (von Sprockhoff, 1955).

A soluble noninfectious complement-fixing BDV antigen may be found in the supernatant by centrifuging infected rabbit brain at 25,000 G's. For diagnostic purposes, this is not done. A 10 percent homogenized brain suspension centrifuged at 3,000 r/min will generally work, with some problems with anticomplementary effect (von Sprockhoff and Nitzschke, 1955; von Sprockhoff, 1956a).

The infected equine brain contains good complement-fixing antigen. The most heavily infected portions of the brain-cerebral cortex, nucleus caudatus, Ammon's horn, and medulla oblongata contained good antigen when 14 of 17 histologically Borna-positive horse brains were positive to the complement-fixation test using rabbit immune serum (von Sprockhoff, 1956b).

A direct immunofluorescent test may be used on the brain of infected animals. Rabbit immune serum is also used for this technique (Wagner and others, 1968; Shadduck and others, 1970).

Wagner and others (1968) used the fluorescent antibody (FA) test on brain impression smears and frozen sections from infected rabbits and one horse. Of six rabbits, one was positive for FA technique while negative to both complement fixation and the presence of JD bodies; in one, lesions were absent but both complement fixation and FA were positive. The remaining rabbits and horse were positive by all three methods.

Shadduck and coworkers (1970) found most fluorescing cells of the infected rabbit brain, concentrated in Ammon's horn, in which the complement-fixing titer was also strongest.

The FA technique is more sensitive than histopathology and at least as sensitive as the complement-fixation test for infected brains. Immunodiffusion has recently been used in experimental studies. This technique shows promise for diagnosis (Ludwig and others, 1973).

SEROLOGY

As yet there is no test for systemic antibodies that can be considered reliable for diagnostic purposes. Nicolau and Galloway (1928) failed in an attempt to find serum-neutralizing antibodies in a rabbit inoculated with and proven immune to challenge exposure to BDV. The same investigators (1930) later found only nonspecific complement-fixing antibody in experimentally inoculated rabbits that reacted with herpes, rabies, and vaccinia antigens.

Von Sprockhoff (1954b), who first developed the high titer rabbit serum used now for the FA and complement-fixation techniques, was unsuccessful in consistently demonstrating complement-fixing antibody in horses with clinical BD.

Fechner (1955) demonstrated complement-fixing antibodies in 130 of 193 infected rabbits. He found antibody in 7 of 10 i.c.-infected and

in one naturally infected horse. Of seven horses vaccinated and subsequently infected, six showed positive titers.

Some infected rats and guinea pigs had high complement-fixing titer (von Sprockhoff, 1957).

Otta (1957) found complement-fixing antibody in horses repeatedly inoculated subcutaneously with virus, but in low concentrations. In three experimentally infected horses, Wagner (1970) found one negative at 41 days p.i., positive at days 43 to 147, and negative at day 151, a second horse showed weakly positive at day 54 and negative again at day 132, and the third horse positive at day 70 p.i. and negative at day 134.

The conclusion to date must be that a positive serum complement-fixation test for BDV is probably significant in equines or in other species, but that negative tests cannot be interpreted. The role of viral-neutralizing antibodies is as yet undetermined because of the lack of, until very recently, a practical indicator system for testing.

LABORATORY ANIMALS

White mice of all ages are apparently refractory to i.c. infection with BDV. In early work, adult mice only (over 20 g) died between 27 and 126 days p.i., while younger animals remained asymptomatic. Dead mice had JD bodies in Ammon's horn (Nicolau and Galloway, 1928). Later several workers (Heinig, 1969) were unable to infect mice of any age with BDV. The resistance of mice to infection would seem to be an important characteristic in primary isolation of this virus for differentiating BD from the equine encephalitides with which it could easily be confused.

The rabbit has been most frequently used for both diagnosis and experimental work. Work with the rabbit has been summarized in other sections. Of additional interest is that virus found in the brains and blood of i.c.-infected rabbits before 10 days p.i. could, in one experiment, be passed only once or twice, while virus found 10 days p.i. and later could be passed indefinitely, suggesting that at the earlier period the virus may be incomplete (Heinig, 1961). Subpassage of virus in lactating rabbits may reduce frequency of JD bodies and other histological lesions (von Sprockhoff, 1956a).

Laboratory rats and guinea pigs are not so susceptible as rabbits to infection but have been used as models since laboratory work began with the virus (Zwick and others, 1926). Rat brain titers for the complement-fixation test have been found to reach 1:32-64 (Nitzschke, 1957). The appearance of JD bodies in rats is more constant than in infected rabbits. Alternate brain passages between rabbits and rats decrease incubation and survival time in rabbits but not in rats. In rats, unlike livestock or rabbits, older animals are more susceptible than the young, and full susceptibility is apparently not reached until 3 months of age (Nitzschke, 1963).

In one experiment, 2 of 10 i.c.-inoculated rats survived to 8 months p.i. with paralytic disorders of their hind legs. When killed at this time, infectious virus was isolated from their brains and virus antibodies demonstrated (Ludwig and others, 1973).

Guinea pigs subjected to i.c. inoculation have great variation in individual susceptibility, with incubation periods ranging from 3 weeks to 13 months. Virus passaged in guinea pigs does not become attenuated for the rabbit (Galloway, 1930). Rhesus monkeys have been susceptible to i.c. inoculation (Nicolau and Galloway, 1928). Of 41 cats inoculated i.c., two died 30 and 44 days p.i. Virus could be isolated from four of the remaining cats when sacrificed 62, 64, 79, and 92 days p.i. (Ihlenburg, 1966).

Results with chicken inoculation have been erratic but artificial infection has been successful (Galloway, 1930; Alkewitz, 1939; Matthias, 1955b; Heinig, 1969). All of 13 i.c.-inoculated day-old chicks showed incoordination motor paralysis of the legs and wings 5 to 8 weeks p.i. Three recovered and two of these tested 1-1/2 years p.i. showed simultaneous infectious brain virus and agar gel precipitin antibodies (Ludwig and others, 1973).

Adult Syrian hamsters inoculated i.c. with BDV showed no symptoms, histopathology, nor systemic antibody up to 309 days p.i. After a variable incubation period, however, virus infectious for rabbits and complement-fixing antigen could be detected from the brains of these animals (Anzil, 1972; Anzil and others, 1973).

Tree shrews (Tupaia glis) are experimentally susceptible to i.c. inoculation of BDV (Ludwig and others, 1973).

VIRUS CULTIVATION

Fertile chicken eggs can grow BDV. Inoculation of the chorioallantoic membrane of 5-day eggs resulted in detection of virus from 6 to 15 p.i. In one trial, 11-day eggs were also susceptible. Originally, adaptation of the virus by alternate rabbit brain passage is apparently necessary. After four rabbit brain-chorioallantoic membrane passages, the virus has been passaged up to 15 consecutive times in eggs. Lowering the temperature to 35° or 35.5° C during the second half of the incubation period seemed to favor virus multiplication. Inoculation of de-embryonated eggs of the yolk sac of fertile eggs was unsuccessful (Nitzschke and Rott, 1957; Rott and Nitzschke, 1958).

Recent success with tissue culture propagation of BDV promises to rapidly increase knowledge of the virus. Mayr and Danner (1972a), in work which they point to BD being a model for slow virus study, infected secondary lamb kidney cultures and passed the virus along with passage of cells.

In one trial, residual infectious virus was present in media and cultures until 72 hours p.i.; it disappeared in both until reappearance 37 days p.i., which proved replication of BDV in the cultures. Sixty-four days p.i. virus was still present in the cultures, at which time passage of the infected cells was done. Both media and cells were positive 34 days post transfer, or 98 days p.i. In other tests, infectivity reappeared in lamb kidney cultures 56 and 76 days postinfection.

Virus inoculation of cultures was at the same time as seeding of the cells. Media was changed every 10 days. The indicator system for virus detection in the media and cells was 6-week-old rabbits inoculated i.c., resulting in characteristic signs, lesions, and presence of complement-fixing antigen 4 to 8 weeks p.i.

Virus-infected lamb kidney cells grew more quickly and became a complete monolayer earlier than control uninfected cells. Ten to 20 days p.i. the monolayers developed foci of enlarged cells with enlargement and vacuolization of the nuclei.

With hematoxylin-eosin stain, Borna-type small compact intranuclear eosinophilic inclusions with halos were occasionally seen after 5 weeks' infection. Occurrence of these inclusions could not be correlated with infectivity of the cells for rabbits. No other cytopathic effect in infected cultures was noted (Mayr and Danner, 1972a).

Borna virus can be maintained for several months by growth and subcultivation of infected rabbit and hamster brain explant cultures. With this technique, rabbit brains were harvested shortly after onset of symptoms, and those of asymptomatic infected hamsters at 122 days p.i. They were subcultured 1 to 2 weeks after preparation and periodically thereafter. Inclusion bodies in infected explanted brain cultures were of the JD type and increased in quantity and size with age of the culture. With immunofluorescence the number of fluorescing nuclei and the size of inclusion-like foci also increased with age of the culture (Mayr, 1972; Mayr and Danner, 1972b). Co-cultivation of GMK (green monkey kidney) cells with those of explanted infected rabbit brain cultures may increase the growth rate of BDV as indicated by increased complement-fixation titers of the co-cultivated cells (Ludwig and others, 1973).

PREVENTION AND CONTROL

The animal health programs of the Federal Republic of Germany were decentralized and reporting of BD is obligatory in those lands where it is endemic--Bavaria, Hessia, and Baden-Württemberg. The German Democratic Republic requires reporting of equine diseases. Vaccination and treatment of animals in all Germany are voluntary (FAO-WHO-OIE, 1970). The efficacy of vaccination is considered good for sheep. There are some doubts about its use in horses.

Control within the endemic zone is based on the premise that the disease

is contagious and that contact with infected feed, water, and fomites may transmit it. The virus is quite resistant to normal environmental influences. It is assumed that there are inapparent carriers of the disease.

In endemic zones it is advised not to keep horses in contact with sheep and cattle, nor to feed and water horses together in large numbers. Sick and suspicious animals are isolated from healthy herds. Disinfection of infected premises is with chlorine or formalin-based disinfectants. Quarantine of horses for at least 2 months before introduction into healthy herds is advised.

No tests of proven value exist to detect the disease in the incubating or inapparent carrier animal. In light of the variability of incubation period in presumably the most direct i.c. infection of sheep and horses, a minimum of 5 months' quarantine of ruminants, equines, and rabbits from known or suspected infected areas into countries free from Borna would seem advisable.

An outbreak of BD in these countries should be combated by drastic eradication procedures.

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